

TREATMENT OR PREVENTION OF DAMAGE DUE TO RADIATION EXPOSURE

BACKGROUND OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATION

5 **[001]** The present application claims the benefit of U.S. Provisional Application
Serial No. 60/488,097, filed July 18, 2003.

1. Field of the Invention

10 **[002]** The present invention relates to the field of the treatment or prevention of
damage due to radiation.

2. Description of the Background Art

15 **[003]** For decades, ionizing radiation has frequently been used as a modality for the
treatment of many types of cancers and tissue abnormalities. Although control of the
delivery of such radiation has improved, the fact that it cannot be precisely controlled in
many areas of the body confers certain unwanted biological side effects, including the
destruction of healthy tissue, radiation burns and sickness, and other similar damage
such as disruption of tissue and cellular architecture, structural changes in cytoskeletal
organization and disruption of the structural organization of actin and various
20 degenerative, immunological, and other injuries to the blood, blood vessels,
microvasculatures, healthy tissues and organs secondary to radiation therapy. In
particular the efficacy of therapy in cancer patients and other patients receiving
radiation treatments is currently limited by the significant damage to surrounding
healthy tissues which includes increased inflammatory responses and the release of
25 toxic intermediates including inflammatory cytokines, chemokines, eicosanoids and
metabolites that limit the effective dose of ionizing radiation in patients.

30 **[004]** Radiation from other sources, including sunlight, gamma rays, X-rays, nuclear
equipment, nuclear facilities, nuclear bombs, "dirty" bombs, high voltage electrical
current, etc., can cause damage, sometimes severe, to tissues of exposed subjects.

30 **[005]** There remains a need in the art for improved methods and compositions for
treating or preventing the damage caused by radiation exposure.

SUMMARY OF THE INVENTION

[006] In accordance with the present invention, a method of treatment or prevention of damage due to radiation exposure comprising administering to a subject in need of such treatment an effective amount of a composition comprising 1) a compound including a radiation damage-inhibiting polypeptide comprising amino acid sequence LKKTET, a conservative variant of LKKTET, an actin-sequestering agent, an anti-inflammatory agent; 2) an agent which stimulates production of said compound in said subject; 3) an agent which regulates said compound in said subject; or 4) an antagonist of said compound, so as to inhibit radiation damage in said subject.

DETAILED DESCRIPTION OF THE INVENTION

[007] In accordance with one embodiment, the present invention relates generally to the treatment, prevention or reversal of physical, cognitive, and biological injuries resulting from exposure to ionizing radiation by the use of the peptide, Thymosin beta 4 (Thymosin $\beta 4$ or T $\beta 4$), or fragments of T $\beta 4$ such as LKKTET, or conservative variants thereof. Sometimes these are referred to as LKKTET peptides or polypeptides. Included are N- or C-terminal variants such as KLKKTET and LKKTETQ.

[008] Over 50% of all cancer patients receive radiation therapy to reduce tumor size. The efficacy of radiotherapy is dose limiting due to the toxic side effects of radiation and the disruption of normal tissue architecture and inflammatory, degenerative and immunological effects to surrounding tissues due either to the direct effects of the x-rays or gamma-rays or to side effects resulting from the release of toxic amounts of tissue and cellular debris from the tumors. As up to 10% of the total protein in tumors is actin and 50% of this protein is sequestered in its monomeric form when the G-actin is released into the blood following destruction of tumor tissues, the physico-chemical properties of the blood induces the polymerization of the G-actin into F-actin, the fibril form of this molecule. This flood of F-actin overwhelms the actin-sequestering properties of the blood and can result in severe pathologies. F-actin alone, when administered to experimental animals, has significant toxicity and is thought to play role in the multi-organ failure, ARDS and other syndromes associated with septic shock. A number of tissues such as the stem cells of the bone marrow, the lymphoid tissues such as the spleen and lymph nodes and the endothelial cells of the gut, have long been known to be highly sensitive to the deleterious effects of ionizing radiation. The deleterious effects on these tissues have previously been attributed to either direct or indirect effects

due to the release of adrenal cortical steroids or to a variety of other additional hormones and growth factors. In addition, the structural disorganization of actin due to direct or indirect effects of radiation is thought to contribute significantly to the toxicities observed. Some of the growth factors which include inflammatory cytokines and chemokines and other agents such as eicosinoids may contribute significantly to the side effects and current limitations of radiotherapy. T β 4, analogs and isoforms and other derivatives, by virtue of their unique properties when administered systemically, locally or topically, are effective in reducing the toxic side effects of radiotherapy. Furthermore, the unique properties of T β 4 include radio-protective effects, thus allowing increased effective doses of radiation therapy. The invention also is applicable to treatment or prevention of damage due to radiation from other sources, including sunlight, x-rays, gamma rays, nuclear equipment, nuclear facilities, nuclear bombs, "dirty" bombs, high voltage electrical current and other sources of radiation.

[009] Without being bound to any particular theory, it is believed that the present invention is based on the discovery that anti-inflammatory peptides and actin-sequestering peptides such as T β 4 and a number of other actin-sequestering peptides which contain the actin binding motif and amino acid sequence LKKTET, are useful for the treatment or prevention of certain biological processes which occur due to exposure to ionizing radiation, and promote treatment or prevention of damage due to ionizing radiation exposure. These peptides have the capacity to promote repair and healing by having the ability to induce terminal deoxynucleotidyl transferase (a non-template directed DNA polymerase), to decrease the levels of one or more inflammatory cytokines and chemokines and to act as a chemotactic and angiogenic factors for endothelial cells, and thus prevent and/or heal and reverse effects that occur due to a number of factors, including exposure to certain x-rays, gamma-rays or other forms of ionizing radiation and radiotherapy of (i) cancer patients, (ii) patients receiving radiation or photo-therapy for skin disorders, or (iii) individuals exposed to acute or lethal doses of ionizing radiation. T β 4 may act as a "rescue molecule", preventing permanent polymerization of actin, preserving the function of actin in cells exposed to radiation and protecting the ability of normal cells to divide. T β 4 may inhibit induction of enzymes which induce apoptosis, thereby inhibiting induction of apoptosis of normal cells which may be caused by radiation. T β 4 may also prevent damage to tissue by modulation of transcription factors associated with improved survival of tissue. T β 4 forms a functional ternary complex with LIM domain protein PINCH and Integrin Linked Kinase (ILK), which are essential for cell survival. T β 4 exposure results in induction, altered

localization and activation of ILK. Formation of a T β 4-PINCH-ILK complex in cells may mediate the protection and/or repair effects of T β 4 independently of actin polymerization. Additionally, T β 4 stimulates the production of laminin-5 in cells which may protect, or facilitate repair of, tissue.

5 [0010] T β 4 was initially identified as a protein that is up-regulated during endothelial cell migration and differentiation in vitro. T β 4 was originally isolated from the thymus and is a 43 amino acid, 4.9 kDa ubiquitous polypeptide identified in a variety of tissues. Several roles have been ascribed to this protein including a role in a endothelial cell differentiation and migration, T cell differentiation, actin sequestration and
10 vascularization.

[0011] In accordance with one embodiment, the invention is a method of treatment or prevention of damage due to ionizing radiation exposure comprising administering to a subject in need of such treatment an effective amount of a composition comprising a radiation damage-inhibiting polypeptide comprising LKKTET, or a conservative variant
15 thereof having radiation damage-inhibiting activity, preferably T β 4, an isoform of T β 4, oxidized T β 4, T β 4 sulfoxide, or an antagonist of T β 4. Administration can be before, during or after exposure of the subject to radiation, so as to protect tissue and prevent damage, and/or salvage and repair tissue.

[0012] Preferred compositions which may be used in accordance with the present
20 invention comprise amino acid sequence LKKTET, amino acid sequence KLKKTET or LKKTETQ, T β 4, an N-terminal variant of T β 4, a C-terminal variant of T β 4, an isoform of T β 4, a splice-variant of T β 4, oxidized T β 4, T β 4 sulfoxide, lymphoid T β 4, pegylated T β 4 or any other actin sequestering or bundling proteins having actin binding domains, or peptide fragments comprising or consisting essentially of the amino acid sequence
25 LKKTET or conservative variants thereof, having radiation damage-inhibiting activity. International Application Serial No. PCT/US99/17282, incorporated herein by reference, discloses isoforms of T β 4 which may be useful in accordance with the present invention as well as amino acid sequence LKKTET and conservative variants thereof, which may be utilized with the present invention. International Application Serial No.

30 PCT/GB99/00833 (WO 99/49883), incorporated herein by reference, discloses oxidized T β 4 which may be utilized in accordance with the present invention. Although the present invention is described primarily hereinafter with respect to T β 4 and T β 4 isoforms, it is to be understood that the following description is intended to be equally applicable to amino acid sequence LKKTET, KLKKTET, LKKTETQ, peptides and
35 fragments comprising or consisting essentially of LKKTET, KLKKTET or LKKTETQ.

conservative variants thereof, as well as oxidized T β 4 and T β 4 sulfoxide, having radiation damage-inhibiting activity.

[0013] In one embodiment, the invention provides a method for healing radiation damage in a subject by contacting an area to be treated with an effective amount of a radiation damage-inhibiting composition which contains T β 4 or a T β 4 isoform. The contacting may be topically, systemically, enterally, by pulmonary delivery, etc. Examples of topical administration include, for example, contacting the skin with a lotion, salve, gel, cream, paste, spray, suspension, dispersion, hydrogel, ointment, or oil comprising T β 4, alone or in combination with at least one agent that enhances T β 4 penetration, or delays or slows release of T β 4 peptides into the area to be treated. Systemic administration includes, for example, intravenous, intraperitoneal, intramuscular or subcutaneous injections, or inhalation, transdermal or oral administration of a composition containing T β 4 or a T β 4 isoform, etc. Enteral administration may include oral or rectal administration. A subject may be a mammal, preferably human.

[0014] T β 4, or its analogues, isoforms or derivatives, may be administered in any effective amount. For example, T β 4 may be administered in dosages within the range of about 0.1-50 micrograms of T β 4, more preferably in amounts of about 1-25 micrograms.

[0015] A composition in accordance with the present invention can be administered daily, every other day, etc., with a single administration or multiple administrations per day of administration, such as applications 2, 3, 4 or more times per day of administration.

[0016] T β 4 isoforms have been identified and have about 70%, or about 75%, or about 80% or more homology to the known amino acid sequence of T β 4. Such isoforms include, for example, T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15. Similar to T β 4, the T β 10 and T β 15 isoforms have been shown to sequester actin. T β 4, T β 10 and T β 15, as well as these other isoforms share an amino acid sequence, LKKTET, that appears to be involved in mediating actin sequestration or binding. Although not wishing to be bound to any particular theory, the activity of T β 4 isoforms may be due, in part, to the ability to regulate the polymerization of actin. For example, T β 4 can modulate actin polymerization in skin (e.g. β -thymosins appear to depolymerize F-actin by sequestering free G-actin). T β 4's ability to modulate actin polymerization may therefore be due to all, or in part, its ability to bind to or sequester actin via the LKKTET sequence. Thus, as with T β 4, other proteins which bind or sequester actin, or modulate

actin polymerization, including T β 4 isoforms having the amino acid sequence LKKTET, are likely to prevent or reduce radiation damage alone or in a combination with T β 4, as set forth herein.

[0017] Thus, it is specifically contemplated that known T β 4 isoforms, such as T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15, as well as T β 4 isoforms not yet identified, will be useful in the methods of the invention. As such T β 4 isoforms are useful in the methods of the invention, including the methods practiced in a subject. The invention therefore further provides pharmaceutical compositions comprising T β 4, as well as T β 4 isoforms T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15, and a pharmaceutically acceptable carrier.

[0018] In addition, other proteins having actin sequestering or binding capability, or that can mobilize actin or modulate actin polymerization, as demonstrated in an appropriate sequestering, binding, mobilization or polymerization assay, or identified by the presence of an amino acid sequence that mediates actin binding, such as LKKTET, for example, can similarly be employed in the methods of the invention. Such proteins include gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, DnaseI, vilin, fragmin, severin, capping protein, β -actinin and acumentin, for example. As such methods include those practiced in a subject, the invention further provides pharmaceutical compositions comprising gelsolin, vitamin D binding protein (DBP), profilin, cofilin, depactin, DnaseI, vilin, fragmin, severin, capping protein, β -actinin and acumentin as set forth herein. Thus, the invention includes the use of a radiation damage-inhibiting polypeptide comprising the amino acid sequence LKKTET (which may be within its primary amino acid sequence) and conservative variants thereof.

[0019] As used herein, the term "conservative variant" or grammatical variations thereof denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the replacement of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the replacement of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

[0020] T β 4 has been localized to a number of tissue and cell types and thus, agents which stimulate the production of T β 4 can be added to or comprise a composition to effect T β 4 production from a tissue and/or a cell. Such agents include members of the family of growth factors, such as insulin-like growth factor (IGF-1), platelet derived

growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta (TGF- β), basic fibroblast growth factor (bFGF), thymosin α 1 (T α 1) and vascular endothelial growth factor (VEGF). More preferably, the agent is transforming growth factor beta (TGF- β) or other members of the TGF- β superfamily. T β 4 compositions of the invention may reduce certain effects of radiation by effectuating growth of the connective tissue through extracellular matrix deposition, cellular migration and vascularization.

[0021] Additionally, other agents may be added to a composition along with T β 4 or a T β 4 isoform. Such agents include angiogenic agents, growth factors, agents that direct differentiation of cells, agents that promote migration of cells and agents that stimulate the provision of extracellular matrix material in tissue. For example, and not by way of limitation, T β 4 or a T β 4 isoform alone or in combination can be added in combination with any one or more of the following agents: VEGF, KGF, FGF, PDGF, TGF β , IGF-1, IGF-2, IL-1, prothymosin α and thymosin α 1 in an effective amount.

[0022] The actual dosage or reagent, formulation or composition that heals damage associated with radiation damage may depend on many factors, including the size and health of a subject. However, persons of ordinary skill in the art can use teachings describing the methods and techniques for determining clinical dosages as disclosed in PCT/US99/17282, supra, and the references cited therein, to determine the appropriate dosage to use.

[0023] Suitable formulations include the inventive composition at a concentration within the range of about 0.001 - 10% by weight, more preferably within the range of about 0.005 - 0.1% by weight, most preferably about 0.01-0.05% by weight.

[0024] The therapeutic approaches described herein involve various routes of administration or delivery of reagents or compositions comprising the T β 4 or other compounds of the invention, including any conventional administration techniques (for example, but not limited to, topical administration, local injection, inhalation, systemic or enteral administration), to a subject. The methods and compositions using or containing T β 4 or other compounds of the invention may be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable non-toxic excipients or carriers.

[0025] The invention includes use of antibodies which interact with T β 4 peptide or functional fragments thereof. Antibodies which include pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody

preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art as disclosed in PCT/US99/17282, supra. The term antibody as used in this invention is meant to include monoclonal and polyclonal antibodies.

5 [0026] In one embodiment, the invention provides a method for treatment or prevention of damage due to ionizing radiation exposure comprising administering to a subject in need of such treatment, an effective amount of a composition comprising a radiation damage-inhibiting polypeptide comprising amino acid sequence LKKTET, or a conservative variant thereof having radiation damage-inhibiting activity.

10 [0027] In one embodiment, the invention provides a method for treatment or prevention of damage due to ionizing radiation exposure in a subject by contacting tissue with a radiation damage-inhibiting amount of a composition which contains T β 4 or a T β 4 isoform. The contacting may be topically, enterally or systemically. Examples of topical administration include, for example, contacting skin or other tissue with a
15 lotion, salve, gel, cream, paste, spray, suspension, dispersion, hydrogel, ointment, or oil comprising T β 4, alone or in combination with at least one agent that enhances T β 4 penetration, or delays or slows release of T β 4 peptides into the area to be treated. Systemic administration includes, for example, intravenous, intraperitoneal, intramuscular or subcutaneous injections, or inhalation (orally or nasally), transdermal,
20 suppository, enema or oral administration of a composition containing T β 4 or a T β 4 isoform, etc. A subject may be a mammal, preferably human.

[0028] The invention provides a method for the prevention and/or healing and reversal of the body, bodily tissues, and organs and/or symptoms associated therewith, resulting from X-rays, gamma-rays or other forms of ionizing radiation and radiotherapy
25 of (i) cancer patients, (ii) patients receiving radiation or photo-therapy for skin or other disorders, or (iii) individuals exposed to acute or lethal doses of ionizing radiation, by the application of a therapeutically effective amount of a composition comprising T β 4, T β 4 analogues, isoforms, or peptide fragments with the amino acid sequence LKKTET and conservative variants thereof.

30 [0029] A method of the invention involves applying a therapeutically effective amount of the composition to a site or systemically on a continuous or periodic basis during a course of therapy to reduce the effects of exposure to ionizing radiation. The duration of administration can range from a single administration to administration for the life of the subject. Preferred courses of administration are in a range of about 1-6
35 months. Administration can be periodic or continuous. During a course of

administration, a composition in accordance with the invention may be administered once, twice, or three or more times per day, and can be administered daily, every other day, every third day, etc.

[0030] According to one embodiment, radiation is administered to a target area of a subject, and a composition in accordance with the invention is administered before, during and/or after administration of the radiation to the target area, so as to inhibit radiation damage in an area of said subject outside said target area.

[0031] A method of the invention involves utilization of a composition which contains an agent that stimulates the production of LKKTET or T β 4 or variants thereof or some other actin-sequestering or anti-inflammatory compound.

[0032] In one aspect of the method, the healing polypeptide is T β 4 or an isoform or oxidized form of T β 4, or a spliced-variant form of T β 4 in a gel, cream, paste, lotion, spray, suspension, dispersion salve, hydrogel or ointment formulation.

[0033] In another aspect of the method the composition may be delivered systemically by injection, orally, nasally, transdermally or any other means.

[0034] The composition may be naturally derived or produced using recombinant methodologies, or other synthetic means such as, but not limited to, solid-phase and solution-phase synthesis.

[0035] One method includes treating exposure to ionizing radiation or other types of radiation in a subject, comprising administering to the subject a composition containing an agent that regulates the actin-sequestering peptide, LKKTET, or T β 4 activity. The agent may be an antibody. The antibody may be polyclonal or monoclonal.

[0036] One method includes ameliorating the toxicity of radiotherapy comprising treating a subject exposed to such radiotherapy with an agent that regulates T β 4 activity.

[0037] In some embodiments, the T β 4 regulating agent is an antisense form or other type of antagonist of T β 4 peptide, or other suitable composition.

[0038] The invention may permit significantly increasing the amount of radiotherapy that a cancer patient can receive by administering an effective dose of T β 4, or T β 4 analogues, isoforms, or other molecules described herein, containing the amino acid sequence LKKTET and other conservative variants that reduce inflammation, and/or actin toxicity, and/or stimulate angiogenesis and protect radio-sensitive stem cells in the blood, bone marrow, gastrointestinal tract and/or other parts of the body.